0932

RESPONSE OF VIRUSES TO ENVIRONMENTAL EXPOSURE *

by

Bailus Walker, Jr., Research Fellow
Environmental Health Research Laboratories
School of Public Health
College of Medical Sciences
University of Minnesota

May 1968

* This review is a part of the continuing research (under NASA contract NGR 24-005-063) on environmental microbiology as related to planetary quarantine.

TABLE OF CONTENTS

LIST OF TABLES

I. INTRODUCTION

Our knowledge about viruses has increased to such an extent in the last decade that it is sometimes necessary to pause and question what the outstanding problems are today. One area of current interest about which there are still large gaps in available information is the effects of environmental stress on the survival of cell-free viruses. This knowledge would have considerable bearing on certain aspects of virus-disease epidemiology, and more recently has stimulated the interest of the National Aeronautics and Space Administration workers who are charged with spacecraft sterilization and planetary quarantine.

It is the purpose of this presentation to review some of the recent developments in this field and to attempt an analysis of the more significant findings.

No attempt will be made to include all of the research findings about the environmental effects on extracellular survival of viruses. Valuable sources of additional information and references can be found in Advances in Virus Research, Annual Reviews of Microbiology, Progress in Animal Virology, and various other symposia and monographs. Similarly, the current techniques for virus isolation, cultivation, titration and identification are adequately described in Diagnostic Procedures for Virus and Rickettsial Diseases, published by the American Public Health Association,[1] and a review by Schmidt and Lennette.[2]

Aspects of deliberate virucidal treatment and inactivation were focal points of the symposium published by the New York Academy of Sciences and will not be elaborated upon here.

The present review will pay particular attention to the natural environment with emphasis on temperature, humidity, light and conditions in

extraterrestrial environments. An attempt will also be made to introduce the subject by considering the physical, chemical and morphological characteristics of viruses, the theoretical rationale underlying virus stability and some aspects of virus disease transmission in the inanimate environment.

II. PHYSICAL, MORPHOLOGICAL AND CHEMICAL PROPERTIES *

Virus are infectious agents which are smaller than the smallest protozoa and bacteria. Although some are barely visible under the light microscope, most lie beyond the range of this instrument and occupy the microbial spectrum between a few millimicrons to several hundred millimicrons.

Diseases of plants and animals that are caused by viruses have long been known clinically, but the existence of viral agents and the elucidation of their characteristics are fairly recent discoveries. Actually, it has been scarcely a centruy since the larger microbes, protozoa, bacteria, yeasts and molds were implicated as causative agents of disease, and since they have been cultured and studied as pure cultures. Among the many well known diseases, however, there were still some which were believed to be infectious, but in which an etiological agent could not be recovered. It was not until the turn of the century that evidence was gathered about a group of entities which did not respond to classical bacteriological detection methods and which lay in a size range even smaller than the smallest bacterium. In 1892, Iwanowski found that the infectious agent of tobacco mosaic could pass through a bacterial filter and induce a disease in a previously healthy plant.

---

* In part from (1) Cohn, J. A. Science, October 20, 1967, pp. 343-350; (2) Luria, S. E. and Darnell, J. E. General Virology, John Wiley and Son, 1967; (3) Smith, Conant, and Overman. Zinsser's Microbiology, 13th Ed., Appleton-Century Croft, 1964.

Beijerinck, in 1899, corraborated Iwanowski's findings, and within a few years many disease agents were found to display this property. The known range of hosts was extended still further when Twort, in 1915, and independently, d'Herelle, in 1917, discovered agents of similar properties which parasitized and destroyed bacteria. More recently agents have been found in this same category which parasitize some of the fungi, an example of which is the actinophage. Such infectious agents came to be known at first as filterable viruses or, with time, simply viruses.

Since the place of viruses in the biological scale has not been fully established, a rigid definition of the agent is not possible. The ability to travel through earthen filters, the criterion first serving to separate viruses from other infectious agents, indicates a smallness of size fully substantiated in subsequent work. A second general differentiating criterion thus far valid is that the agents are obligate parasites multiplying only within living cells.

Constitutionally and physically, viruses vary widely in complexity but, in comparison with other analogous entities such as cells, protozoa and bacteria, are all of extreme simplicity. Nevertheless, the respective agents are definitive biological units manifesting the fundamental property of reproduction. Except for this capacity, the smallest and the simplest virus approaches closely the essential nature of large molecules. In fact, in some definitions the agents have been designated as nucleoprotein macromolecules, but this is an unwarranted simplification detracting from full appreciation of the total spectrum of viral characteristics.

In their morphological aspects, viruses are particulate entities which occur in a large variety of shapes and sizes. Many virus causing diseases in plants and insects are rods of highly symmetrical shape and uniformity of size.

Generally the viruses of man and animals are spherical in shape. Some of the smaller viruses are so uniform in shape and size that they can be caused to aggregate into fairly large perfect crystals. When this occurs, it is possible to observe their ordered structure, either by photographing a replica of their surfaces or a plane cut through their interiors--or by photographing a thin section cut from them after fixation and embedding.

The application of freeze-drying and critical point techniques have revealed that many viruses are distinctly polyhedral in shape. The heads of all frozen-dried bacterial viruses examined are so shaped. Many of the plant viruses are, and even virus of the insect Tipula paludosa is, exquisitely polyhedral. The importance of this discovery is its implication that a virus is synthesized from subunits that fit together in an ordered fashion to produce a particle that is, in itself, at least partially crystalline.

Constitutionally viruses exhibit a principal feature in common; namely, these agents contain only protein and nucleic acid. They differ strikingly, however, in the kind of nucleic acid and in the arrangement of nucleic acid and protein. Ribonucleic acid only is a component of all plant viruses, some animal disease agents and a few bacteriophages, whereas other simple animal viruses contain only DNA. A major advance has been the demonstration that the protein component is not a collection of different protein molecules but consists of an orderly arrangement of identical protein molecules or subunits. Studies of tobacco mosaic virus with carboxypeptidase resulted in the liberation of only one amino acid, threonine, which thus showed that this amino acid was the terminal group of the polypeptide chains constituting the protein components. Complete analyses have revealed the kind and number of other amino acids constituting the individual protein molecule. The total number of amino acids per polypeptide chain was 158. Such intensive studies have not

been made with rod shaped viruses, but morphological evidence indicates that those examined exhibit the same subunit structure and are thus similar in principles of protein constitution to tobacco mosaic virus.

Nucleic acid analyses have yielded information of relatively equal significance for all viral agents. From morphological and other evidence it would appear that nucleic acid, whether DNA or RNA, is virus-specific and represents no additives of host cell material. In consequence, the amount and kind of nucleic acid is a specific viral characteristic regardless of nature of assembly or adventitious enveloping or associated substances. The proportions of nucleic acid vary greatly from one agent to another; however, a different perspective emerges with calculations of the absolute amount of nucleic acid per individual virus particle. It then becomes evident that the nucleic acid content of most RNA viruses varies little, approximately the value of 2×10^6 molecular weight. The lowest value, 1.5×10^6, is that of tobacco necrosis, and the highest in the comparable range is 3.1 for foot and mouth disease. A notable exception to the relative uniformity is the BIA strain A avian tumor agent with a molecular weight of 9.6×10^6. A like value was obtained for the Rous sarcoma virus.

The nucleic acid of viruses occurs either as double-stranded DNA arranged in the Waston-Crick double spiral, as a single-stranded DNA or as RNA. The molecular size may vary from as few as one million to as many as 130 dalton units. It is likely that DNA is double-stranded in most DNA agents, as in the rabbit papilloma virus. In the X174 bacteriophage, in contrast, DNA seems to be single-stranded. This single-stranded DNA of noncomplimentary base composition is present in a circular form. The long rod-shaped phages M-13 and fd, specific for male E. coli, also contain single-stranded DNA. Like the DNA of X174, the molecular weight is relatively low, between 1.5 and 2×10^6 daltons.

It has been shown that initiation of the infectious process and specific virus synthesis of some agents can be induced by nucleic acid alone. This was accomplished first with RNA isolated from tobacco mosaic virus and subsequently with the RNA of a variety of both plants and animal viruses. It had been realized for some time that only DNA of the tailed bacteriophages entered the parasitized bacterium. Recognition of the phenomenon was extended more recently to DNA animal viruses and of the papova group, rabbit papilloma and polyoma agents, for example, and the infection is transmitted also by the single-stranded DNA of the X174 bacteriophage.

Such findings effect more clarification of the functional significance of nucleic acid composition and provide the basis for judgment relative to the role of auxiliary components of proteins and lipids. It would appear that the material encasing nucleic acid serves two principal functions: (1) partly to influence specificity of host-cell parasitization, and (2) to protect nucleic acid during exposure to adverse environmental conditions inside or outside the cell. It is unlikely that transmission by free nucleic acid is a significant factor under natural conditions, and nucleic acid must be conserved in the process of transfer from one cell to another and also under varied conditions of virus uptake by the cell. In some cases nucleic acid alone can make its way into the cell, but in others whole virus particles are taken up. The influence of virus membrane or sheath on specificity of host cell infection is indicated by the broader spectrum of hosts parasitized more by nucleic acid than by the whole virus.

III. THEORETICAL CONSIDERATION OF VIRUS STABILITY

The stability of virus particles exposed to various environments is a "chemical stability," that is, a static kind of stability. To alter a virus particle, its structure must be changed. The situation is somewhat different

from the one encountered, for example, in the study of the stability of bacteria. In the case of bacteria we are dealing with "organismic stability," that is, with a dynamic system in a state of continuous flux into which and from which component elements are continuously incorporated, assimilated, eliminated and broken down. In such a system irreversible inactivation may occur following rather mild environmental changes, since any disturbance of the dynamic situation may lead to lack of protection against new situations produced by the organism's own activity. For example, a virus and a bacterium may be equally sensitive to a lowering of the pH. To inactivate the virus with acid, acid must be added. The bacterium, however, may be killed by a change in buffer concentration which allows it to accumulate enough acid from its own metabolic activity to bring the pH down to the toxic level. Consequently, studies on bacterial stability are more analogous to the situation of virus stability when it is inside its host cell, than to the stability of viruses in an extracellular environment.

Changes in the nature of virus particles brought about by external stress can be classified into three major categories: reversible inhibition, inactivation without loss of antigenicity, and disintegration. Inhibition of infectivity of many viruses by a variety of substances, including proteins, enzymes, and plant and insect extracts, has been described repeatedly.[3,4,5] Such nonlethal changes are typified by the reproductive delays produced in bacteriophages by small doses of radiation and resulting from chemical treatment of tobacco mosaic virus. Very few virus changes have been described that are not accompanied by some loss of activity.

Apparently a true inactivation of a virus particle that is a complete destruction of infectivity and capacity of multiplication must include an irreversible change in its nucleic acid. Until recently, inactivation of the

nucleic acid was generally considered to be a one-hit, all-or-none phenomenon, referable to essential sites in the molecule. Most probably, however, the concept of particular chemical groups as carriers of the biological activity represents an oversimplification of the problem. It seems more reasonable to regard activity as an expression of a complicated pattern of forces, determined by the structure of the molecule as a whole. Any particular site is essential only as part of the pattern, and any alteration, physical or chemical, is important only insofar as it significantly modifies the field of forces. For example, an amino acid group might be essential only because by means of an H bond, it maintains the whole polymer in a given stereochemical pattern. A substitution of one H atom might lead to instability or inactivation, not because maintenance of the intact NH_2 group is vital but because the reaction may break an H bond, which in turn may bring the amino acid group out of line and distort the specific folding pattern of the molecule.(6)

With this reasoning, inactivation as a two- or multiple-hit phenomenon may be easily envisaged. If, for example, two groups are linked by a double H bond, breakage of both bonds may be needed for disruption of the linkage. Generally, the results will depend not only upon the point of attack, but also upon the nature of the environmental stress.(7)

Inactivation without disintegration of the virus particle is generally effected by treatments that do not cause extensive protein denaturation. Radiation and mild treatments with chemicals such as formalin of hydrogen peroxide often produce this result. Protracted or intense exposure to an agent that at first causes only a loss of activity and may lead to extensive denaturation and disintegration and often causes a change in shape and morphology of the virus particle. Occasionally several properties of a virus can be suppressed one after the other by progressive exposure to one or more

unfavorable environmental conditions. The properties that are destroyed first are probably those that require the greatest degree of structural integrity and are, therefore, suppressed even by a slight chemical alteration. The ability to reproduce is generally lost very soon, whereas antigenicity is very stable, apparently requiring for its suppression a far reaching alteration of the virus proteins.[8]

As previously indicated, viruses can be dissociated into their constituent nucleic acid and proteins. The extracted nucleic acid largely free of protein coat is in itself capable of initiating infection but with considerable reduced efficiency. This finding, in turn, led to the discovery[9] that the separated RNA of poliovirus, for example, can infect cells of species that had long been considered to be completely insusceptible to infection. Parenthetically, findings such as these have prompted the suggestion put forth by Herriott[10] that extracellular virus may sometimes exist in the native state as naked nucleic acid devoid of its usual protein coat. The state of nucleic acid capable of inducing infection and virus replication has not been clarified in all cases. It is known, however, that the total tobacco mosaic virus RNA strand is required to transmit infections and that a single break in the chain destroys infectious capacity.[11] Less definitive results with RNA animal viruses have also suggested the requirement for a complete chain for infectiousness.

Inactivation of free nucleic acid has not been studied sufficiently as yet to permit any profitable discussion of the nature of its response to various stresses of the natural environment. As already pointed out however, the NA is normally encased in a protein cover. In effect, therefore, it is separated from the surrounding medium by a semipermeable membrane. A chemical agent, for example, can act upon the NA only if it is able by one way or

another to penetrate this membrane. Under these conditions the extent of virus stability obviously will depend on the rate of penetration. A chemical may be capable of penetration either because of its small molecular size or because of its capacity to break down the protein. With the exception of certain enzymes, the molecular size of which excludes the possibility of penetration, few substances are sufficiently specific to react exclusively with NA and not at all with proteins. Therefore, it is a logical conclusion that an inactivating agent modifies the protein as well as the NA of the virus. Whatever the type of the reaction, changes in charge and hydration of the protein are to be expected which, together with the structural alterations, will affect its permeability. In other words, in the course of exposure to an agent which affects the stability of the virus, the rate of penetration of the protein coat hardly can be expected to remain constant, and the extent of destabilization of the NA will vary accordingly.

Theoretically, a gradual breakdown of the protein should tend to increase the permeability of the membrane, the consequence being acceleration of inactivation. This phenomenon might be expected in acid or alkaline hydrolysis of the viruses. The opposite effect, a gradual increasing stability, is to be expected in reactions with tannings or hardening agents causing fixation of the protein in the histological sense.

With regard to thermal stress, there is little knowledge available about the thermal expansivities of proteins and nucleic acid. There is some indication that heat produces basic structural alterations of the virus particle due to differential expansion of various viral components. Some early measurements that were auxillary to sedimentation studies indicated that protein expansion coefficients are high. Pollard[12] has made some measurements on egg albumin and showed that a value of 1.6×10^{-4} per degree centigrade is

reasonable. Ribonucleic acid does not seem to expand nearly as much. The protein expansivity is almost certainly due to some change in secondary structure as the large value would require 90 per cent (according to Pollard) of all bonds to be hydrogen bonds; this is certainly not the case. Consequently, some kind of unfolding must occur. Possibly the unfolding cannot occur in nucleoprotein, but, at the same time, internal tensions may develop that have the effects of destroying the functioning of the virus.

Some physical agents act on various molecular units and alter their function. As an example, nucleic acid and, in a secondary way, proteins are both sites of action of ultraviolet light. In both cases, the quantum yield is not very high and, in the case of nucleic acid, the mechanism of which is not clearly understood, could be caused by two factors: (1) cross-linking of the nucleic acid, and (2) breaking of the nucleic acid chain, thus preventing it from producing the specific ribonucleic acid for protein. In the case of protein, the effect is somewhat more clearly understood. It seems likely that one of the major effects of ultraviolet light is to cause a S-S bond to become excited, thus rendering it capable of being broken by the action of water or possibly in some other way. Such a rupture can be followed by the denaturation of the protein which will change its configuration and cause it to cease functioning properly. Similarly, radiation acts upon both the nucleic and the protein. The fact that very frequently the sensitivity of the virus corresponds physically to the sensitivity of the nucleic acid probably reflects the greater importance of the nucleic acid in the virus function than that of the protein.

The effects of pressure on viruses can be divided into two classes. Where the exposure is to extremely high pressure ranging up to many thousands of atmospheres, there is a totally disruptive effect on the virus that is

reflected in a loss of activity. Of greater interest is the fact that at lower pressures the relationship to thermal inactivation begins to appear. Thus if a virus is heated while under pressure, the pressure may stabilize the virus. It would seem as though the critical bond that is necessary for the inactivation of the virus may well have a definite increase in stability because of the fact that every part of the molecular structure is held closer while the pressure is exerted, resulting in a clear stabilization.[13] Clearly then, the effects of any environmental agent are influenced by a composite of conditions (composition of medium, etc.). Sometimes it is difficult to decide which factor is actually responsible for the observed virus change, since a number of conditions all present together may cause more damage than would the sum of their individual effects.

IV. VIRUS DISEASE TRANSMISSION AND ENVIRONMENT

Since they are obligate parasites, viruses cannot multiply outside of a susceptible host. Rather, in this cell-free state, they must survive despite a multitude of adverse environmental influences of physical and chemical nature, inactivating ultraviolet or other radiation, variations in temperature and slow dessication. Indeed, relative vulnerability to environmental forces while in the free state is an important factor in transmission.[13] Consequently, a review of virus epidemiology might provide some insights into the enigma of virus survival. Very little epidemiological data exist to verify the significance of airborne viruses.

Unfortunately, elucidating the influence of environment on virus infections is inherently complex and often impossibly difficult. Potentially relevant environmental factors are both numerous and of many types, physical chemical and biological. Also in any given instance, these factors are

operating simultaneously, and the task of evaluating their individual contribution is very great. Further, many environmental factors contribute only by indirect and sometimes almost circular paths.(13)

It might be appropriate at this point to define the concept of "inanimate environment" with respect to virus disease transmission. Although classical epidemiology recognizes six transmission pathways (direct contact, indirect contact, droplet contact, vectors, vehicles, and airborne) only three of these pathways qualify as part of the inanimate environment: indirect contact via inanimate fomites; inanimate vehicles such as food, water and milk; and airborne as droplet nuclei or attached to dust. In each of these cases, the virus spends sufficient time in a cell-free state between reservoir and host to be influenced by environmental stress. In the other three cases, the virus remains as an intracellular parasite, and the epidemiology of those infections tell us little about the response of agent to environmental exposure.

The mechanism and extent of viral disease transmission also depend on the salient features of the natural histories of the particular infections. Some viruses such as polio, measles and mumps are exclusively parasites of man. A few, such as dengue, are believed to parasitize only man and invertebrate vectors. Others, including yellow fever and St. Louis encephalitis viruses, depend on lower vertebrates and invertebrate vectors or, as in the case of rabies and ornithosis, on lower vertebrates alone. Whether the vertebrate host be man or lower vertebrate, the main pathways for invasion of and escape from the host are respiratory, alimentary, percutaneous or mucosal. Invasion and escape often, but not invariably, utilize the same pathways. Also, whatever the vertebrate host, its role as a source of infection is usually limited to a brief period following onset of the infection, but may be recurrent in association with recrudescence--or indefinitely persisting. Finally, vertical

transmission from generation to generation, in either vertebrate or invertebrate host, may contribute to the reservoir mechanism.[14,15]

Common usage not withstanding, a viral reservoir is not a vertebrate or invertebrate host species. More precisely, it is the total mechanism that assures survival of the virus species and, even when infection persists for the life of an individual host, takes the basic form of a continuing chain of transmission from vertebrate host to vertebrate host, with or without the intervention of an invertebrate host as a biological vector. Thus, a functioning reservoir requires the existence of susceptible host in sufficient abundance under conditions that assure continuing transmission.

For the most part, the individual host is an effective source of infection for a relatively brief period only, and the vital chain is formed of many short links. Assuming that infection is followed by resistance to reinfection of significant degree and duration, persistence of a particular virus in a circumscribed population requires a continuous input of enough susceptibles. Excluding special cases of rapid population turnover, as exemplified in military recruit populations and even a hospital population, this input will be supplied by newly born infants and reentry into susceptible state of persons whose immunity has worn off. The adequacy of this input should be a function of the rate of effective contact and of viral infectivity and duration of post-infection immunity which, for a given virus, should be constant.[13]

That infection does not always lead to disease is an epidemiologic principle of first importance. The reasons why disease results in a given instance may relate to the virus (highly pathogenic strain), to the host (genetic susceptibility, age nutritional state), or to the subject of our present concern, the environment. How environmental forces influence the outcome of infection is the specific question and one for which there would seem

to be three general answers: (1) by altering the disease-producing properties of the agent, (2) by influencing host susceptibility, and (3) by acting, after infection has been established, to influence its course.

Airborne transmission is intended to mean transfer of infection by means of small particle aerosols.[16,17] These particles are evaporated residues of infected respiratory secretions which are of such small size that they will remain airborne for long periods of time. Environmental factors may determine the immediate and long-term survival of the cell-free agents; may dictate the mechanism by which man is exposed and becomes infected; may govern the frequency and duration of exposure; and when infection does occur, may exert some influence on its outcome, for example, silent or overt infection, trivial or serious disease.[13]

The concept that respiratory viruses are transmitted by the airborne route has been popular in the past, primarily because it seemed reasonable to assume that coughing and sneezing, common symptoms of viral respiratory disease, produced aerosols that would accomplish such transmission. In this respect, it is necessary to clarify the distinction between droplet infection and true airborne transmission. In the former case, reservoir and host exist in close proximity to each other, and the virus in the sneeze or cough droplet is never really "aware" that it is in an inanimate environment. In the latter case, the virus travels for considerable time or distance between reservoir and host and is literally airborne. The fact that infected persons are capable of producing airborne virus does not necessarily indicate that virus can be transmitted in this way. Viral aerosols produced by infected persons are subject to dilution in air, biological decay and sedimentation.[18]

Many very labile viruses usually avoid running the environmental gauntlet by exploiting an intermediate invertebrate host as a biologic vector. For

those viruses which do travel from infected to susceptible hosts without such protection, the length, duration, and nature of the path of transmission tends to reflect the stability of the virus. Such labile viruses as measles, respiratory syngitial and the parainfluenzas which invade the respiratory portals via droplets are not noted for long-range airborne spread or serious environmental contamination. This is in sharp contrast to smallpox which matches measles for infectivity but, as shown in the New York City episode in 1947,[19] can strike at great range (from the first to the fourth floor of a hospital) and also can cause persisting environmental contamination. Increased stability enables viruses to employ more indirect, though mechanical modes of spread. Epidemic keratoconjunctivitis and pharyngoconjunctivities caused by adenoviruses may be spread via welders' goggles in shipyards or the water of swimming pools.[13] Poliovirus and, presumably, most other hardy enteroviruses are quite susceptible to drying but can persist over long periods in flies, in sewage, and in sewage polluted waters, and so when environmental hygiene is lacking, may presumably be spread by a multitude of indirect paths. Particularly impressive for its apparent resistance to chlorination is the virus of infectious hepatitis. In Delhi, India, in late 1955, severe flooding reversed the current of a river so that the sewage outfall was temporarily upstream from the water supply intake. Chlorination was successful in controlling all sewage-borne, viral, bacterial, and higher parasites except hepatitis virus which caused upwards of 35,000 cases.[20]

The obviously great dependence of transmission on environmental influences suggests that appropriate environmental changes may be parallelled by corresponding changes in viral transmission. With respect to contact transmission (defined to include short-range droplet spread), the important changes in the physicochemical environment are related to temperature and humidity, and these

have been invoked to explain influenza and poliomyelitis and summer diseases respectively in Holland.[21] Deliberate changes such as aerosol disinfectants, ultraviolet radiation and greatly increased exchange of air so far offers uncertain promise as methods for control.

All things living, viruses included, are subject to mutation which occurs spontaneously, presumably due to "copying errors" in the replication of the DNA or RNA source of genetic information, or which may be induced by chemical (sulfonates, guanidine, nitrous acid) or physical (UV, x-ray) means.[13]

For agents such as the enteroviruses or hepatitis virus which may persist in the free state in waters polluted by industrial wastes, one can envision induced mutation as a somewhat greater possibility. How an environmental influence may select particles with differing pathogenic potential is illustrated with polioviruses in cell culture at high ($\pm$ 40° C) or low ($\pm$ 40° C) temperatures, "hot" strains lending to have increased neurovirulence for monkeys, and cold strains the converse.[22] Under seminatural conditions (vaccine strains multiplying in the intestinal tracts of febrile children), this same influence could not be demonstrated, but environmental selection of mutant strains with modified disease potential remains plausible.

V. TEMPERATURE AND HUMIDITY EFFECTS

The information available about environmental effects on viruses is different from that available for other microorganisms because it has been gathered by investigators guided by different practical purposes. The practical goals of survival and/or inactivation studies on viruses have been the preservation of virus activity in the laboratory, the separation of infectivity from seriological activity for the production of vaccines, and the choice of suitable manipulations in the handling of viruses for purification

and concentration. A more distant goal has been the possible application of inactivating agents for suppression of virus infectivity.

One of the most significant studies conducted on the effects of environmental factors on viral agents is that of Harper[23] in which he measured the influence of relative humidity (R.H.) on the survival of four viruses: vaccinia, influenza, Venezuelan equine encephalomyelitis (VEE) and poliomyelitis. Tests with poliomyelitis were carried out between 21 and 24° C; the other viruses were examined at this and two additional temperature ranges: 7 - 12° C and 32 - 34° C. The viability of vaccinia and influenza virus was high one second after dissemination, indicating that these viruses were not affected by the existing environmental conditions. The highest per cent recovery of VEE and poliomyelitis virus one second after dissemination was obtained at approximately 85 per cent relative humidity. Recovery of viruses was reduced with a decrease in relative humidity indicating a dependency of viability on humidity. The influence of temperature on viable decay in stored aerosol was similar for all three viruses (VEE, vaccinia and influenza;

TABLE 1

Viability of Airborne Virus 0-23 Hours After Spraying[23]

Temp. (°C)	R.H. (%)	No. of Tests	Percentage viable at given times (hr.) 0*	1/12	1/2	1	4	6	23
			(a) Vaccinia						
10.5-11.5	20	1	94	68	78	82	79	81	66
	50	1	94	90	90	83	92	77	59
	82-84	2	97	81	71	79	59	60	27
21.0-23.0	18-19	2	97	86	80	66	46	45	15
	48-51	3	93	82	83	86	57	50	12
	82-84	3	112	96	73	66	24	18	Trace
31.5-33.5	17-19	2	80	67	67	61	51	33	13
	50	2	74	76	68	51	26	15	Trace
	80-83	2	88	88	54	36	5.9	1.2	Trace

(Table 1 - cont'd)

Temp. (°C)	R.H. (%)	No. of Tests	0*	1/12	1/2	1	4	6	23
				(b) Influenza					
7.0-8.0	23-25	3	88	87	80	78	68	63	61
	51	3	66	49	75	61	39	42	19
	82	3	126	120	71	70	39	35	3.0
20.5-24.0	20-22	5	75	77	65	64	74	66	22
	34-36	3	86	93	58	59	66	53	14
	50-51	3	84	62	49	29	6.4	4.2	Trace
	64-65	3	77	45	29	15	6.6	3.2	N.D.
	81	4	67	55	22	13	6.4	5.0	Nil
32.0	20	3	87	70	56	45	18	17	1.3
	49-50	3	98	45	22	13	2.7	0.7	Nil
	81	3	91	50	15	6.6	Trace	Trace	Nil
				(c) Venezuelan equine encephalomyelitis					
9.0-9.5	19	1	69	54	25	20	27	50	26
	48	1	100	86	16	24	29	24	11
	86	1	105	90	57	119	100	67	6.2
21.0-23.0	19-23	2	23	17	19	14	11	7.5	1.7
	50	2	35	28	21	14	7.8	5.2	0.1
	81-86	2	92	63	82	26	16	4.0	0.1
32.0-33.0	19	2	27	25	18	9.9	6.9	3.1	0.17
	48	2	25	22	8.5	6.1	ca. 1.0	0.1	Trace
	81-85	2	132	80	33	17	ca. 2.0	Trace	Nil
				(d) Poliomyelitis					
20.5-23.5	18-23	4	19	10	5.5	5.9	3.4	3.3	1.1
	35-36	4	19	7.4	6.4	6.7	5.6	5.3	0.9
	49-51	5	66	0.6	0.16	0.06	0.03	Trace	Trace
	64-65	5	96	96	91	94	61	55	10
	80-81	5	120	131	112	124	111	105	85

* Samples collected ca. 1 sec. after spraying.

N.D. = not done.

Trace = Samples containing viable virus in amounts too small for accurate assay.

poliomyelitis was tested at only one temperature) All survived better at the lowest temperature tested. In this respect the viruses behaved like most bacteria so far examined.

With influenza and poliomyelitis viruses, the influence of R.H. was examined in more detail in the temperature range 20-24° C. There was a sudden increase in the viable decay rate of influenza virus when the R.H. was raised above 35 per cent. At a R.H. of 50, 65 and 80 per cent, viable decay rate of this virus proceeded at closely similar rates. However, with poliomyelitis virus, where the influence of R.H. was reversed, the sudden increase in viable decay rate when the R.H. was lowered to 50 per cent was followed by improved survival at R.H. of 35 and 20 per cent, though viable decay rates were still more rapid than at R.H. above 50 per cent. The data in Table 1 indicate that the airborne particles remain viable for a considerable time in favorable conditions and that these conditions are not the same for the four viruses tested.

Another significant piece of research is that of Ehrlich, Miller and Idoine.[24] These investigators used bacteriophage as a model system in their experimental studies to determine the recovery levels and the decay rates of coliphage aerosolized at different humidities from fresh and stored cultures. They also examined the effects of selected compounds on the survival of T-3 coliphage at adverse humidity conditions. The initial aerosol stability of the coliphage, calculated as per cent recovery two minutes after aerosolization, was not affected by prior storage in nutrient broth containing 5 g per liter of sodium chloride at 10 or -70° C for up to four months. However, a slight increase in the subsequent aerosol decay rate of the coliphage that had been stored at 10° was observed (Table 2).

TABLE 2

Effects of Storage on Viability of T-3 Coliphage Aerosolized at 85% Relative Humidity and 23° C [24]

Lot #	Mean Culture Count plaque/ml			Aerosol Recovery at 2 min.			Aerosol Decay Rate per min.		
	Fresh	Storage 4 mo.		Fresh	Storage 4 mo.		Fresh	Storage 4 mo.	
		10° C	-70° C		10° C	-70° C		10° C	-70° C
				%	%	%	%	%	%
Pool	81×10^8	34×10^8	5.4×10^8	46.2	66.3	57.8	5.5	8.5	6.6
1	33×10^8	21×10^8	12×10^8	55.8	79.4	46.8	5.5	6.5	5.3
2	78×10^8	75×10^8	16×10^8	68.4	58.8	52.4	5.5	6.3	4.6
3	72×10^8	54×10^8	$.07 \times 10^8$	70.9	55.9	57.6	5.6	6.6	6.7
4	86×10^8	43×10^8	1.4×10^8	65.6	65.2	50.2	6.4	7.4	4.4
5	114×10^8	65×10^8	26×10^8	47.9	58.1	49.9	4.9	8.0	8.3

The survival of airborne coliphage was also investigated at 30, 55 and 85 per cent relative humidity (Table 3). Statistical analysis of the average per cent recoveries and aerosol decay rate indicated that the observed differences were significant. The highest decay rate occurred at 55 per cent relative humidity. The highest initial aerosol recovery was obtained at 85 per cent relative humidity.

TABLE 3

Effects of Humidity on Viability of Airborne T-3 Coliphage [24]

Relative Humidity %	Aerosol Recovery at 2 Min. %	Aerosol Decay Rate/Min. %
85	55.0	5.0
55	13.1	12.8
30	.13	9.7

Turning to the effects of different chemicals on coliphage aerosol stability, these investigators incorporated various additives in the phage suspension before the particles were aerosolized. Compared with untreated controls the additives had no material effect upon the initial stability of the aerosols at 50 and 85 per cent relative humidities. However, 0.1 M concentrations of dextrose seemed to retard subsequent biological decays, at least at 50 per cent relative humidity. The aerosol decay rate of the coliphage at 50 per cent relative humidity in the presence of 0.1 M dextrose was 3.6 per cent per minute, as compared with 8.5 per cent per minute for coliphage disseminated without the additive. The difference was significant. The aerosol decay rates of these two treatments at 85 per cent relative humidity were not significantly different.

In a similar study, Webb[25] reported on survival and inactivation of airborne pigeon pox and Roux sarcoma viruses at relative humidities ranging from 10 to 100 per cent, with and without the addition of inositol. Pigeon pox virus was not affected by changing the relative humidity during the five hours of aerosol life. At relative humidities about 50 per cent, the Roux sarcoma virus was fairly stable, but inactivation increased with a decrease in relative humidity until 30 per cent relative humidity, below which recovery of the live virus increased. When the virus was aerosolized from a 6 per cent inositol solution, the decay under 70 per cent relative humidity was reduced. With both viruses, the inactivation was most rapid during the first hour of aerosol life, and there was no indication that the decay rate was reduced by inositol during this period.

Songer[26] designed an experiment utilizing a modified toroid for a static aerosol chamber to study the effects of relative humidity and temperature on airborne viruses. Studies were conducted at 23° C and at three relative

humidity levels (10, 35 and 90 per cent) with four viruses: Newcastle disease virus (NDV), infectious bovine rhinotracheitis virus (IBR), vesicular stomatitis virus (VSV), and Escherichia coli B-T3 bacteriophage (T-3 phage). At 23° C airborne NDV and VSV survived best at 90 per cent relative humidity. All of the viruses had the poorest survival at 35 per cent relative humidity. At 10 per cent relative humidity, NDV survived equally well at 23 and 37° C.

Data from several studies, including Songer's, tend to show some relationship between sensitivity to relative humidity and other characteristics of viruses. In Table 4, eight viruses are listed with their sensitivity to relative humidity, their nucleic acid core, their sensitivity to ether and their classification, as suggested by Wilner.[27]

TABLE 4

Comparison of the Sensitivity of 11 Viruses to Generation and Storage at Three Relative Humidity Levels[26]

Virus	RH Level Favoring Survival			Nucleic Acid Core	Ether Sensitivity	Classification by Wilner	Ref.
	Low	Med	High				
Roux sarcoma			+	RNA	+	myxovirus	25
Influenza A	+			RNA	+	myxovirus	23
Newcastle Disease	+			RNA	+	myxovirus	26
Pigeon Pox	+	+	+	DNA	+	poxvirus	25
Vaccinia	+			DNA	chloroform+	poxvirus	23
Poliomyelitis			+	RNA	+	picornavirus	23
Psittacosis			+	RNA/DNA	+	not classified	28
Bacteriophage T-3			+	DNA	-	bacteriophage	26

Although these data are incomplete, they suggest that simple generalizations cannot be made regarding the sensitivity of different groups of viruses to relative humidity. Some ribonucleic acid (RNA) viruses survive best at low

relative humidity, and others at high humidity. Some viruses that survive best at high relative humidity are ether-sensitive, and others are not. One of the myxoviruses survives best at high relative humidity; the other two at low relative humidity. Sensitivity to relative humidity appears to be an individual characteristic of a virus.

Additional data are presented by Akers and colleagues[29] who recently studied three strains of Columbia SK group viruses (Mengo, Maus Elherfeld (ME) and Col-SK viruses) to determine if the three strains are identical in aerosol behavior. All three strains were found to give identical aerosol decay patterns at 16 or 26° C when held at the same relative humidity. During the first five minutes of aerosol storage time at 16° C, virus inactivation was relative humidity-dependent, with survival maximal at either high (greater than 80 per cent) or low (less than 5 per cent) relative humidity. After five minutes at 16° C, further inactivation, regardless of relative humidity, was insignificant. At 26° C, the effect on survival of relative humidity between 40 and 60 per cent was even more pronounced than at 16° C, and continued after five minutes through six hours. Results of this study indicated that the inactivation of airborne Col-SK group viruses was similar to that of other ribonucleic acid (RNA) viruses, particularly polio. Since members of the Col-WK group are picornaviruses, they may well serve as an aerosol model representative of small, ether-resistant, single-stranded RNA viruses.

Of considerable epidemiological significance are the findings of Sidwell and co-workers.[30] They investigated the persistence of vaccinia virus on a variety of wool and cotton fabrics. The fabrics were contaminated with virus either by direct contact, by aerosol, or by dust and were then stored at low and high humidities at constant temperatures. Eighty sterile swatches of each fabric were exposed to 10^9 cell culture 50 per cent infectious doses ($CCID_{50}$)

of virus by one of the three methods. The fabrics were then placed in the humidity cabinets, and five swatches of each fabric were tested for the presence and titer of virus at the following time intervals: immediately after exposure to virus (zero-time), 2 hours, and 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 weeks after virus exposure or at least two time intervals after virus could no longer be recovered from the material. The virus titers of the individual swatches varied relatively little from the mean liter of virus recovered, regardless of the manner of exposure of the swatch to the virus; 95 per cent confidence limits were usually less than $\pm$ 0.5 log. Each method of exposure of the swatches to virus resulted in approximately constant amounts (10^8 to 10^9 $CCID_{50}$) of agent being recoverable at zero-time. In most cases, however, the persistence of the virus varied with the method of exposure. For example, at 35 per cent humidity virus placed on cotton fabrics and on wool blanket material, as a dust, was recovered in higher titer than when placed on fabrics as an aerosol or by direct contact. At this same humidity, virus applied as an aerosol generally persisted longer than liquid virus suspensions pipetted onto these same fabrics. At 78 per cent humidity, the results were more varied. The virus generally could not be recovered in two to four weeks from wool fabrics and in one to two weeks from cotton fabrics held in the high humidity. It was observed that the virus remained viable on the wool materials for longer periods of time than on cotton fabrics. The virus appeared to be less stable in the high humidity, and the method of exposure of the fabrics to virus apparently had an effect on the persistence of the agent. On all fabrics, viral persistence was of sufficient duration to be of epidemiological significance.

A related report by Dixon[31] describes the persistence of poliovirus on wool and cotton fabrics exposed to the agent by the same three methods

described previously, after storage of the virus-contaminated fabrics in high and low relative humidities. When held at 35 per cent relative humidity, virus persisted for twenty weeks on wool fabrics, but only one to four weeks on cotton fabrics. At this relative humidity, virus titers on wool fabrics decreased rapidly to low but detectable levels which persisted for long periods of time, whereas in 78 per cent relative humidity the decrease in virus titer was less rapid, but the period of viral persistance was shorter. Generally, virus titers on cotton fabrics held in both relative humidities decreased exponentially to an undetectable level. The type of fabric and weave apparently influenced the recovery of the agent, and again the method of fabric contamination had a profound effect on the persistance of the virus. However, the decrease in virus titer with time on any given fabric was consistent regardless of which method of contamination was used. Poliovirus persisted on wool material for the longest periods of time, substantiating the results obtained by Sidwell[30] with the vaccinia virus studies. An explanation for these observations is difficult, and one is tempted to speculate that certain types of fabrics may provide a protective environment for the virus. Wool fibers consist mainly of keratin and have a cuticle of overlapping scales. Cotton fibers are flattened, twisted cellulose tubes with a small amount of pectins and waxes in the outer wall. The natural moisture content of wool is somewhat higher than that of cotton. There is also the possibility that the virus is held less tightly to the woolen fabrics than to the cotton material, thus allowing the virus to be recovered in higher titer. However, since the virus titer decreased, in most cases, at a steady rate with increasing time, the implication is that the agent lost its viability upon storage.

Greene[32] systematically investigated the stability of thirteen non-pathogenic bacteriophages, representing five different host related groups,

which were compared to T-1 phage with regard to submicron aerosol stability and resistance to desiccation. He found a wide spectrum of stability between phage groups, between individual strains of a homologous group and within the same phage strain after purification. Less significant differences were noted between the drying treatments (Table 5). T-1 coliphage, for example, behaved alike in the crude and semipurified state (after sephadex filtration), but was considerably more labile after dialysis; the same was true with B-3 (pseudomonas phage). On the other hand, another pseudomonas phage (D-3) was more labile after sephadex filtration than before and, in the case of a bacillophage (NR-2), neither sephadexing nor dialyzing exerted any marked influence.

TABLE 5

Survival of Phages After Drying on Surfaces[32]

(Log Ratio Reductions)

Phage		Crude			Sephadexed			Dialyzed		
	Trials	Static		Airblast	Static		Airblast	Static		Airblast
		<2hrs.	>8hrs.		<2hrs.	>8hrs.		<2hrs.	>8hrs.	
T1	(38)	1.66	2.22	1.88	1.02	2.82	2.38	5.37	6.50	6.18
MSP-8	(14)	0.20	0.26	0.16	-	0.81	-	-	-	-
MSP-2	(13)	0.18	0.42	0.28	0.39	-	0.51	-	-	-
B-3	(24)	0.35	0.57	0.68	0.81	0.67	0.68	2.16	5.95	3.83
F-116	(13)	-	1.13	1.33	-	2.51	1.72	-	-	-
D-3	(16)	0.35	0.47	0.79	1.18	1.65	1.62	-	-	-
E-79	(1)			1.79						
WR-2	(26)	2.18	1.13	1.31	1.72	1.10	1.93	2.18	-	2.18
WR-1	(6)	4.26	4.68	4.06						
80	(1)			1.22						
81	(11)	1.11	1.06	1.40	0.95	2.83	1.69			
3A	(6)	0.48	1.96	1.64						

Probably the most significant single variable influencing aerosol stability other than the phage type itself was the menstrum from which the phage was nebulized. This influenced not only the ultimate particle size (which in turn would affect the physical decay rate), but also the amount of protection the naked virus would have during drying, the degree of clumping, the osmotic pressure in the droplet, and the chemical denaturation of virus protein during denaturation.

Aerosolization techniques can also influence apparent aerosol stability. The obvious effect is by generating either large or small original droplets and thus influencing the rate of drying and ultimately the rate of biological decay. A more subtle effect involves the stresses to which the virus is exposed in the generator reservoir. Greene (op cit) found that some phages were partially inactivated by these stresses, whereas at least one showed an apparent increase in count. Warren and Hatch[33] reported similar findings in a study of prolonged storage and aerosol stability of T-3 coliphage. Fresh suspensions of phage were examined for stability in aerosols or were held as long as 72 days at a variety of temperatures and storage conditions and then were aerosolized. The aerosol studies were carried out in rotating drums held at 21° C and varied relative humidities. Phages surviving the initial stress of aerosolization became inactivated at similar rates regardless of the relative humidity, except at a very low level (10 per cent R.H.) where they were inactivated rather quickly. The stored phages were found to survive better at -20° or 21 to 31° C than at 4° C. Agitation or wide fluctuation in temperature during storage, however, was detrimental. In general, storage treatments did not markedly affect aerosol stability of these phages. Rafajko and Young[34] carried out studies to provide information on the thermal as well as the pH stability of adenovirus Type 12, 14, and 18. They found that these

viruses were relatively stable when exposed to 4 and 37° C. At 56° C, Types 12 and 18 were completely inactivated, and Type 14 was more than 99 per cent inactivated after eight minutes. Thirty successive freeze-thaw cycles caused no loss of infectivity of Types 12 and 18. Type 14 showed no inactivation after 15 cycles.

A similar study has been performed by Sharp and his collaborators[35] who demonstrated that vaccinia virus is exceedingly stable with an indicated half life of "many" years at -72° C. In this experimental work vaccinia virus preparations were kept in a Revco mechanical refrigerator at -62° C for 171 days. They were titrated weekly for four weeks and then at longer intervals. In a series of ten titrations, there was no detectable change in virus quality. One preparation was subjected to three successive cycles of freezing at -20° C and thawing in a water bath at room temperature. Titration results and particle counts indicated that this virus was not measurably altered by this treatment.

VI. LIGHT SENSITIVITY

Since Raab[36] first reported in 1900 the destructive effects of visible light on microorganisms as mediated through photosensitizing dyes, considerable attention has been given to the deliberate inactivation of viruses by this method. But the information is scanty on the effects of visible light in the absence of photosynthesizing dyes.

One of the earliest systematic investigations of this subject was by Skinner and Bradish[37] who made a comparative study of the influence of exposure to light on the infectivity of suspensions of viruses of (a) foot and mouth disease (FMD), (b) vesicular stomatitis (VSV), (c) influenza, (d) Newcastle disease (NDV), and (e) fowl plague. Suspensions were prepared by grinding infective tissue in the proportion of 1 g to 9 or 24 ml. of suspending

medium. After clarification by centrifugation the supernatant was taken as the initial virus suspension. The light intensity to which suspensions were exposed during operations was normally about 300 footcandles and never exceeded 600 footcandles. Exposure of the virus suspensions to daylight was accomplished by making a single dilution of the initial virus suspension and distributing it as two 5 ml. samples in screw capped bottles. One of these bottles was exposed to daylight on a laboratory bench in front of a window facing west and the other kept in the dark to serve as a control. Approximate average light intensity during the study period (April-August) thirty inches from window facing west, for the period 10:00 a.m. to 2:00 p.m., was 400 footcandles when the sky was cloudless and 180/300/700 footcandles when the sky was completely overcast with low/medium/high clouds. In view of this variable intensity of daylight, the investigators employed a source of light of constant intensity when comparison was to be made between different experiments.

The infectivity titers of the VSV, influenza NDV and fowl plague followed by exposure to daylight for four hours were 3-5 logarithmic units lower than those indicated by the dark controls. Inactivation due to exposure to daylight was demonstrated in similar experiments with an egg strain of vaccinia virus, a mouse neurotropic strain of influenza virus and with guinea pig strains and mouse neurotropic strains of virus of vesicular stomatitis. Losses of infectivity increased with the intensity of illumination and with duration of exposure. The infectivity of FMD virus was relatively stable during exposure to light.

Although significant inactivation was demonstrated under the conditions described, the data obtained indicated that many factors influence the extent to which a given virus might be affected by light exposure. Where consideration is limited to a single strain of one virus, known factors which may influence the susceptibility to inactivation are the source and nature of the virus.

Cutchin and Dayhuff[38] demonstrated that measle virus in the fluid state was rapidly inactivated by exposure to visible light. They also showed that there is a relationship between the rate of inactivation, the intensity of illumination and wavelength. It appears that the shorter the wavelength, the more rapid is virus destruction

TABLE 6

Influence of Wavelength on Light Inactivation of Measles[38]

Exposure Time (minutes)	Filter					
	450 mu		500 mu		550 mu	
	Light	Dark	Light	Dark	Light	Dark
0	4.7*	-	4.7	-	5.5	-
30	3.5	-	4.7	-	5.5	-
120	2.5	4.7	4.5	4.5	>4.5	5.1

*Log_{10} TCD_{50}/ml

To determine whether photoinactivation under intense illumination was characteristic of measle virus only, poliovirus, Type 3, and vaccinia were compared with that of measles.

TABLE 7

Influence of Artificial Light on Measles, Poliovirus, Type 3, and Vaccinia Virus[3

Exposure Time (minutes)	Measles		Polio, Type 3		Vaccinia	
	Light	Dark	Light	Dark	Light	Dark
0	4.5*	-	7.2	-	7.5	-
10	3.7	-	-	-	-	-
20	3.2	4.7	7.7	7.5	7.5	6.7
30	2.5	-	-	-	-	-
40	1.5	-	-	-	-	-
50	<1.0	-	-	-	-	-
60	<1.0	4.2	7.7	7.7	6.5	7.2

*Log_{10} TCD_{50}/ml

As seen in Tables 6 and 7, measle virus was no longer infectious after fifty minutes of exposure whereas infectivity of poliovirus and vaccinia with initial titers of $10^{7.2}$ and $10^{7.5}$ TCD_{50}/ml, respectively, was essentially unaffected by this treatment.

Additional evidence of light sensitivity of viruses is in the work of Nemo and Cutchins[39] who studied canine distemper virus and found that it was also inactivated by visible light sensitive both in vitro and in vivo.

VII. EXTRATERRESTRIAL EFFECTS

Over the past few years extensive interest and activity have developed in defining the extraterrestrial environment and its influence on the viability of microorganisms. Much of the information, to date, has come from speculation and extrapolation of terrestrial laboratory data from experiments with bacteria, specifically the Bacillus species.[40,41] The main factors of concern have been high vacuum, ultraviolet radiation, x-radiation, cosmic rays and temperature extremes. Several reports have discussed the effects of these environmental factors on bacteria, and most of the pertinent references are included in two recent reports published by the National Academy of Sciences.[42,43] However, only a very few studies have been concerned with virus and its response to the environmental stresses of space.

Kalter and collaborators[44] reported one of the first successful direct exposures of terrestrial viruses to the environment of space and their recovery. Laboratory strains of influenza (PR8) and ECHO 1 virus were flown on board of Discoverer XXIX and XXX. Both flights were similar regarding their flight profile: launch date, recovery, time in orbit, maximum acceleration, and duration. Good agreement (Table 8) was obtained between test and controls (original virus preparation maintained in the laboratory at -20° C) with both viruses. While these flights were not a critical test for space conditions,

TABLE 8

Effects of Discoverer XXIX Flight on Influenza PR8 and ECHO 1 Viruses[44]

Virus	Virus Titer		
	Test	Ground Control	Laboratory Control
PR8	10^{-5}	10^{-5}	10^{-5}
ECHO 1	$10^{-5.3}$	$10^{-5.3}$	$10^{-5.3}$

because the van Allen belt was not penetrated, the data indicate that the viruses did withstand the rigours of space flight. Thus, vibration, temperature fluctuations, alteration in force, and weightlessness produced by these conditions did not alter the stability of the virus utilized in the experiment. Since virus function was not altered, maintenance of functional structure apparently is not impaired by zero gravity. The maintenance of virus structure tends to indicate that gravitational forces (maximum acceleration was 9.0 g) as encountered do not contribute appreciably to the stability of intermolecular binding, at least for the two viruses considered. Unfortunately, no radiation stress sufficient to alter the virus particle was encountered. However, as indicated by the authors, it is recognized that large radiation dosages are necessary before any effect on the virus particle can be detected by present techniques.

The work of Hotchin[45] provides additional evidence of the survivability of virus in space. Two flight experiments were conducted: one using a rocket for short-term exposure at high altitude; the other using a balloon for longer exposure at lower altitude. The samples consisted of sterile surfaces of autoclaved nylon-reinforced "millipore" filter disks for the collection of microorganisms during flight and also additional surfaces coated with dried

preparations of various terrestrial microorganisms, including poliovirus, Type 3 (wild), and Escherichia coli bacteriophage T-1. Nylon-reinforced "millipore" filter membranes (450 mu porosity), each approximately 1 cm^2 in area, were cemented to lucite plates and autoclaved. Aqueous suspensions (consisting of the appropriate culture media) of the viruses were prepared, and 0.1 ml volumes were spread on these surfaces. The exposure began at an altitude of 80 km, and the rocket reached a height of 155 km at apogee.

TABLE 9

Virus Titers in Rocket Experiment by Hotchin[45]

Virus	P.F.U.* Seeded	Ground Set		Flight Set	
		Shielded	Exposed	Shielded	Exposed
T-1 bacteriophage[1]	9×10^6	8.3×10^6	2.9×10^6	2.6×10^6	1×10^2
Polio, Type 3[2]	1.5×10^7	5.3×10^3	3.1×10^3	3.3×10^1	0

*P.F.U. = plague forming units

[1]Titrated at the end of the experiment after recovery, 15 days after launch, and 26 days after preparation of dried disk.

[2]Titrated 22 days after launch and 33 days after preparation of the dried disk.

Twenty six days after inoculation, the ground set phage viable count showed an insignificant loss due to drying: In the case of the ground set poliovirus, the loss was much greater since the dried viable count fell by a factor of 10^4. There was no significant difference between shielded and exposed portions of either virus. The shielded sample of T-1 phage of the flight unit showed a viability not significantly different from the controls, whereas the unshielded viable count had fallen by a factor of 10^4. The viable poliovirus fell by a factor of 10^2 in the shielded flight sample, and by a factor of at least 10^3 in the exposed sample to a level below the threshold of

the assay system which was 16 infective virus particles per cm^2 of surface.

In the balloon experiment the phage loss of the control was much greater. The six hour exposure at 34 km reduced the titer of the exposed flight by a factor of only 10, compared with the shielded set.

Results of laboratory studies to determine the nature of the primary cause of inactivation by environmental factors indicate that vacuum, low temperature, and cosmic rays did not represent significant lethal factors for T-1 phage. The poliovirus showed definite loss of viability in the shielded sample, possibly due to high temperatures.

It is known that intensities of ultraviolet light and of soft x-ray are considerably diminished at balloon altitude (34 km) through absorption at higher atmosphere. The greater survival of the samples during the long-term exposure on the balloon seems to reflect the greatly reduced flux of the ultraviolet light and soft x-ray at balloon flight altitudes. The results of the laboratory experiments indicate that T-1 phage was not screened by the millipore filter membrane from ultraviolet light of wavelengths close to 253 $\mathring{A}$ (as mainly emitted by germicidal lamps) even though the average pore size was much greater than the phage head diameter. In more recent works, Hotchin and associates[46] measured the survival of viruses in space exposed on Gemini XII Satellite. T-1 coliphage, poliomyelitis, tobacco mosaic virus, canine hepatitis, bovine rhinotracheitis influenza (PR8) and vaccinia virus were exposed to space for six hours and twenty-four minutes on Gemini XII Satellite in freeze-dried three dimensional matrices on metal plates in a remotely controlled box outside the satellite. Subsequent assays showed that substantial survival had occurred with all organisms except poliovirus and rhinotracheitis. The results indicated that certain viruses are very resistant to the space environment if provided with a minimum of protection against lethal UV radiation.

It appears from these studies that the ability of the viruses to survive space flights give further indication of the need for additional studies on problems associated with the role of viruses as an environmental health hazard in future space travel, as well as in the implementation of planetary quarantine requirements.

VIII. CONCLUSION

This review necessarily has been limited to a few of the highlights of studies of the response of cell-free virus to environmental exposure.

In recent years investigations requiring the study of bacteria in the environment have undergone a marked expansion, as evidenced by publications in the journals of appropriate disciplines and by symposia. This has been less true of cell-free virus and there is a need for more information on these agents.

The theoretical bases for the influences exerted by response to the physical environment by cell-free virus seems, on cursory inspection, rather simple. A particular environmental stress may act directly on the viral agent by influencing its dissemination either by affecting the mechanism of transmission or by determining the frequency or duration of exposure. However, it has become progressively more evident that the role of environmental factors in the survival of viruses and also in determining disease occurrence is not only very extensive but understood only in rather superficial ways as, for example, in relation to viral spread. Remaining problems of first importance relate to a clear definition of environmental parameters affecting the survival of the viral agents, the alteration in the pathogenic potential of virus and the emergence of new viral agents which may respond differently to environmental stress, when compared with the "old" or more similar viruses.

Despite our limited "knowledge", the phenomenology of environmental virology is of considerable importance to the National Aeronautics and Space Administration's Planetary Quarantine program. Questions about the efficacy of sterilization treatment for bacteria can be fairly raised and should be answered with respect to viruses not only on the basis of assumptions or extrapolation but supported by at least minimal experimental data. Similarly, questions already answered about bacterial survival on different space hardware surfaces should be also asked about viruses.

IX. LITERATURE CITED

1. Diagnostic Procedures for Virus and Rickettsial Diseases, 2nd Ed. 1956. American Public Health Association, New York, N.Y.
2. Schmidt, N. J. and E. H. Lennette. 1961. Prog. Med. Virology 3:1.
3. Ross, A. F. and W. M. Stanley. 1938. The partial reactivation of formalized tobacco mosaic virus protein. J. Gen. Physiol. 22:165.
4. Kliger, I. J. and E. Elenick. 1943. Inactivation of phages by aldehydes and aldoses and subsequent reactivation. J. Immunol. 47:325.
5. Lauffer, M. A., W. C. Price and A. W. Petre. 1949. The nature of viruses. Adv. Enzymol. 9:171.
6. Gard, S. 1960. Theoretical considerations in the inactivation of viruses by chemical means. Ann. N.Y. Acad. Sci. 83:638.
7. Glasstone, S. 1940. Textbook of Physical Chemistry. D. Van Nostrand Company, Inc., New York, N.Y.
8. Rivers, T. M. and F. L. Horsfall, Jr. 1959. Viral and Rickettsial Infections of Man, 3rd Ed. J. B. Lippincott Company, Philadelphia, Pa.
9. Holland, J. J., L. C. McLaren and J. T. Syverton. 1959. The mammalian cell-virus relationship, IV. Infection of naturally unsusceptible cells with enterovirus ribonucleic acid. J. Exp. Med. 110:65.
10. Herriott, R. M. 1961. Infectious nucleic acids, a new dimension in virology. Science 134:256.
11. Schramm, G. 1961. Perspectives in Virology 2:38.
12. Pollard, E. C. 1961. Theory of the physical means of the inactivation of viruses. Ann. N.Y. Acad. Sci. 83:654.
13. Fox, J. P. 1966. The environment and the virus. Arch. Environ. Health 1:91.

14. Fox, J. P. 1951. The emergence of new viral and rickettsial disease New Orleans Med. Surg. J. 104:103.

15. Fox, J. P. 1960. Virus infection and human disease past, present and future. J. Mississippi Med. Assoc. 1:71.

16. Davies, C. N. 1960. Deposition of dust in the lungs; a physical process. Industrial Pulmonary Disease, pp. 44-58. E. J. King and C. M. Fletcher (eds), Little Brown & Co., Boston, Mass.

17. Hatch, T. R. and P. Gross. 1964. Pulmonary deposition and retention of inhaled aerosols. Academic Press, Inc., New York, N.Y.

18. Couch, R. B., T. R. Cate, R. G. Douglas, P. J. Gerone and V. Knight. 1966. Effects of inoculation on experimental respiratory viral Disease in volunteers and evidence of airborne transmission. Bacteriol. Rev. 3:517.

19. Wernstein, I. 1947. An outbreak of smallpox in New York City. Amer. J. Public Health 37:13-76.

20. Melnick, J. L. 1957. A waterborne urban epidemic of hepatitis. Hepatitis Frontiers. F. W. Hartman (ed), Little Brown & Co., Boston, Mass.

21. Hemmes, J. H., K. C. Winkler and S. M. Kool. 1962. Virus survival as a seasonal factor in influenza and poliomyelitis. Antonie Leeuwenkoek 28:221.

22. Sabin, A. B. 1961. Reproductive capacity of polioviruses of diverse origin at various temperatures in the Gustav Stern Symposium on Perspectives in Virology, Vol. 6, Burgess Publishing Company, Minneapolis, Minnesota. Pollard, M. (ed).

23. Harper, G. J. 1961. Airborne microorganisms survival test with four viruses. J. Hygiene 59:479.

24. Ehrlich, R., S. Miller and L. S. Idoine. 1964. Effects of environmental factors on the survival of airborne T-3 coliphage. Appl. Microbiol. 12:479.

25. Webb, S. J., R. Balker and R. W. Hodges. 1963. The effects of relative humidity and inositol on airborne viruses. Can. J. Microbiol. 12:479.

26. Songer, J. R. 1967. Influence of relative humidity on the survival of some airborne viruses. Appl. Microbiol. 15:35.

27. Wilner, B. I. 1965. A classification of the major groups of human and lower animal viruses, 2nd Ed. Cutter Laboratories, Berkeley, California.

28. Rosebury, T. 1947. Experimental airborne infection. The Williams & Wilkins Co., Baltimore, Md.

29. Akers, T. G., S Bond and L. J. Goldberg. 1966. Effects of temperature and relative humidity on survival of airborne Columbia SK Group viruses. Appl. Microbiol. 14:361-364.

30. Sidwell, R. W., G. J. Dixon and E. McNeil. 1966. Quantitative studies on fabrics as disseminator of viruses, I. Persistence of vaccinia virus on cotton and wool fabrics. Appl. Microbiol. 14:55.

31. Dixon, G. J., R. W. Sidwell and E. McNeil. 1966. Quantitative studies on fabrics as disseminator of viruses, II. Persistence of poliomyelitis virus on cotton and wool fabrics. Appl. Microbiol. 14:183.

32. Greene, V. W. 1966. Stability of bacteriophage aerosols. (Final Report to U.S. Army Biological Center, Maryland). School of Public Health, University of Minnesota.

33. Warren, J. C. and M. T. Hatch. 1968. Prolonged storage and aerosol stability of T-3 coliphage. Bacteriol. Proc. American Society for Microbiology, Ann Arbor, Michigan.

34. Rafjaki, R. R. and J. C. Young. 1964. Thermal and pH stability of adenovirus. Proc. Soc. Exp. Biol. Med. 116:683.

35. Sharp, D. G., Sadhukhan and G. J. Glasso. 1964. The slow decline in quality of vaccinia virus at low temperatures (37° C to 62° C). Proc. Soc. Exp. Biol. Med. 115:811.

36. Raab, O. 1900. Veher die wirkung fluorescieren-der stoffe auf infusorien. Z. Biol. 39:524.

37. Skinner, H. H. and C. J. Bradish. 1954. Exposure to light as a source of error in the estimation of the infectivity of virus suspensions. J. Gen. Microbiol. 10:377.

38. Cutchin, E. C. and T. R. Dayhuff. 1962. Photoinactivation of measle virus. Virology 17:420.

39. Nemo, G. J. and E. C. Cutchins. 1966. Effects of visible light on canine distemper virus. J. Bacteriol. 91:789.

40. Hagan, C. A., E. J. Hawrylewicz and R. Erlich. 1964. Survival of microorganisms in a simulated martian environment. Appl. Microbiol. 12:215.

41. Young, R. S., P. H. Deal, J. Bell and J. L. Allen. 1963. Bacteria under simulated martian conditions. Ames Research Center, Moffet Field, California. Paper for Committee on Space Research Symposium, Warsaw, Poland.

42. National Academy of Sciences, 1966 a. Biology and the exploration of Mars. Publication 1296. C. S. Pittendrigh, W. Vishniac and J. P. T. Perlman (eds), Washington, D.C.

43. National Academy of Sciences, 1966 b. Extraterrestrial life: an anthology and bibliography. Publication 1966A. E. A. Shneour and E. A. Ottesen (compilers), Washington, D.C.

44. Kalter, S. S., A. Hellman, G. T. Daye and H. W. Jordan. 1962. Stability of virus on Discoverer Flight XXIX and XXX. Nature 196:13.

45. Hatchin, J., P. Lorenz and C. Hemenway. 1965. Survival of microorganisms in space. Nature 206:442.

46. Hotchins, J., F. Baher and L. Benson. 1968. Survival of viruses in space exposed on Gemini XII Satellite. Bacteriol. Proc. American Society for Microbiology, Ann Arbor, Michigan.